AD NUMBER
AD355396
CLASSIFICATION CHANGES
TO: unclassified
FROM: secret
LIMITATION CHANGES
TO: Approved for public release, distribution unlimited
FROM: Distribution authorized to DoD and DoD contractors only; Foreign Government Information; 16 MAR 1964. Other requests shall be referred to British Embassy, 3100 Massachusetts Avenue, NW, Washington, DC 20008.
AUTHORITY
DSTL, WO 189/415, 30 May 2008; DSTL, WO 189/415, 30 May 2008

SECRET

AD 355396L

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA

SECRET

P.T.P. 900

P.T.P. 900

PORTON TECHNICAL PAPER No. 900

THE INHIBITION OF THE LIVER MICROSOMAL N-DEMETHYLATION OF MORPHINE AND T.2636 BY N-ALLYL NORMORPHINE AND ITS PHARMACOLOGICAL IMPLICATIONS. [U]

BY

D.R. DAVIES and L. LEADBEATER.

CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT
Porton Down, Salisbury, Wilts.

SECRET

SECRET

PORTON TECHNICAL PAPER 900
DATE: 16.3.64

THE INHIBITION OF THE LIVER MICROSOMAL N-DEMETHYLATION OF MORPHINE AND T2636 BY N-ALLYL NORMORPHINE AND ITS PHARMACOLOGICAL IMPLICATIONS

by

L. Leadbeater and D. R. Davies

SUMMARY

Theories of the pharmacological action of morphine imply that the N-demethylation of morphine by liver microsomal enzymes should be competitively inhibited by N-allyl normorphine (Nalorphine). These have been challenged on the grounds that Nalorphine is a non-competitive inhibitor.

The inhibition of morphine and T2636 N-demethylation by Nalorphine has been investigated using rat liver microsomes and it appeared, from the application of the method of Lineweaver and Burk, to be neither competitive nor non-competitive but a mixture of the two. Nalorphine was shown to be metabolised approximately twice as fast as morphine by the same microsomal preparations, i.e. in the inhibition studies the inhibitor concentration was not constant, hence the simple Michaelis-Menton theory (on which the method of Lineweaver and Burk is based) cannot be applied to this system. It is concluded that this type of experiment may be used neither to support nor challenge hypotheses of the pharmacological activity of morphine.

(Sgd.) D.R. Davies,
Head, Biochemistry Section.

LL/DRD/JMO

(Sgd.) W.S.S. Ladell,
Assistant Director (Medical).

SECRET

PORTON TECHNICAL PAPER 900
DATE: 16.3.64

THE INHIBITION OF THE LIVER MICROSOMAL N-DEMETHYLATION OF MORPHINE AND T2636 BY N-ALLYL NORMORPHINE AND ITS PHARMACOLOGICAL IMPLICATIONS

by

L. Leadbeater and D. R. Davies

INTRODUCTION

N-allyl normorphine (Nalorphine) antagonises the pharmacological effects of morphine (1) and inhibits the liver microsomal enzyme system which N-demethylates morphine in vitro. It has not been demonstrated that the antagonism of the pharmacological effects by nalorphine is caused by inhibition of morphine N-demethylation in the CNS. However, these two inhibitory actions of nalorphine have been related in two theories of the mechanism of morphine action by Beckett, Casy and Harper (2) and by Axelrod (3).

Beckett, Casy and Harper (2) have suggested that analgesia is only produced after the N-demethylation of morphine at the receptor sites in the brain. Nalorphine, these authors believe, has a greater affinity for such sites than morphine and hence antagonises morphine action by displacing it from the site. In effect Beckett et al. believe that nalorphine is a competitive inhibitor of morphine N-demethylation.

Axelrod (3) showed that the chronic administration of morphine to rats depressed the N-demethylating activity of their liver microsomes towards morphine. This treatment also produced tolerance to the pharmacological actions of the drug. He also found that nalorphine, given together with morphine, protected the enzyme from inactivation. Nalorphine alone, given chronically to rats, depressed the demethylating activity of the microsomes and produced a tolerance to morphine (4). When morphine and nalorphine were given together the depression of microsomal demethylation and the degree of tolerance fell between the values for the compounds given individually.

Axelrod (3) therefore suggested that "the interaction of narcotic drugs with the demethylating enzymes inactivates the enzymes. Likewise, the continuous interaction of the drugs with their receptors may inactivate the receptors".

This implies that the N-demethylating enzymes of the liver microsomes may serve as a model for the drug receptor sites and if this is true nalorphine should be a competitive inhibitor of microsomal N-demethylation.

These hypotheses have been criticised by Way and Adler (5). One of their grounds of criticism is a report by Axelrod and Cochin (6) that nalorphine is a non-competitive inhibitor of morphine N-demethylation. More recently Elison, Elliott, Look and Rapoport (7) have confirmed this finding by two separate methods. They stated that their results failed to support either Beckett's theory or Axelrod's theory and concluded that the liver microsomal enzymes are not suitable as models by which these theories may be judged.

T2636 is a potent analgesic whose pharmacological effects are antagonised by nalorphine (8). In a recent study in which the biochemical and pharmacological properties of morphine and T2636 were compared (9), the kinetic experiments of Axelrod and Cochin (6) and of Elison et al. (7) were repeated. The results failed to confirm that nalorphine is a non-competitive inhibitor. From the present data the inhibition appears to be a mixture of competitive and non-competitive and it is concluded that the hypotheses of Beckett et al. and of Axelrod may be neither supported nor challenged on data obtained in these experiments.

METHODS

Microsomal Enzyme Preparation

Five adult male albino rats of the Porton strain were used for each preparation. The animals were killed and their livers excised, as quickly as possible, and placed in ice-cold 0.10 M Tris-HCl buffer (pH 7.50), in an ice-bath. The livers were dried between filter paper and dropped into a 100 ml cylinder containing 50 ml buffer and their volume determined. The liver was then homogenised in 2 volumes of buffer in a 500 ml head of the M.S.E. Atomix blendor for 10 seconds at full speed. All these manipulations were carried out in a cold room (0-3°C) using pre-cooled apparatus and solutions. The homogenate was centrifuged in a refrigerated Model L Spinco Centrifuge in the No.30 head (stored in the cold overnight) for 20 minutes

at 12,000 r.p.m. The microsomal enzymes, contained in the supernatant together with the soluble fraction of the liver cell, were assayed immediately after preparation.

Incubation Conditions

A modification of the incubation medium of Axelrod (10) was used:-

Microsome plus soluble fraction equivalent to 250 mg liver	
NADP	4×10^{-5}M
Nicotinamide	10^{-2}M
Semicarbazide HCl (Adjusted to pH 7.5)	2×10^{-2}M
$MgCl_2$	5×10^{-3}M
Tris-HCl Buffer (pH 7.50)	5×10^{-2}M

The incubations were carried out in a total volume of 5.0 ml contained in a 50 ml tube which was shaken in air at 37.5°C.

The rates of metabolism of morphine, T2636 and nalorphine were linear for at least 30 minutes. For the inhibition studies the N-demethylation of morphine and T2636 was estimated from the amount of formaldehyde produced in 20 minutes. The relative rates of metabolism of nalorphine and morphine were determined by estimating the amount of normorphine formed during a 30 minute incubation. The rates of metabolism are expressed as μmole/g liver/hour (it was assumed that the Specific Gravity of liver tissue is 1.0).

Estimation of Formaldehyde

Formaldehyde was determined colorimetrically as diacetyldihydrolutidene by the method of Cochin and Axelrod (4).

Estimation of Normorphine

Normorphine was assayed colorimetrically as its cupric dithiocarbamate derivative using a modification of the method described by Umbreit (11) for the estimation of secondary amines in the presence of tertiary and primary amines.

4.0 ml of a solution made up of 35 ml carbon disulphide, 25 ml pyridine and 65 ml iso-propanol were added to the 5.0 ml incubation medium, followed by 2.0 ml 0.0013 M cupric chloride in 50% (W/V) pyridine. The tube was shaken vigorously for 1 minute. Exactly 15 minutes later 3.0 ml 10% (W/V) acetic acid and 6.0 ml benzene were added and the tubes shaken again for 2 minutes. The tubes were centrifuged and 1.0 ml of the benzene layer was diluted with 4.0 ml 20% (W/V) isopropanol in benzene. The optical density of the solution was measured at 440 mμ in a Unicam SP 500 spectrophotometer. Standard curves were obtained by putting known amounts of normorphine through the procedure.

RESULTS

Four different preparations of microsomes, each isolated from the pooled livers from five rats, were used in these experiments. The rates of N-demethylation of morphine and T2636 were determined at various substrate concentrations in the presence and absence of 3×10^{-4} M nalorphine and the data fitted to the Lineweaver and Burk equation (12). The substrate concentrations were chosen so that the points were uniformly distributed along the $^1/S$ axis. The equation of the best fitting line was determined by the method of least squares and the values of Km (Michaelis Constant) and V (maximum velocity of the reaction) calculated from the equations. The significance of the difference between the values was determined by the "Student" T test (13).

(1) The Inhibition of Morphine N-Demethylation

The results for the inhibition of morphine metabolism are shown in Table 1.

TABLE 1

The Inhibition of Morphine N-Demethylation by Nalorphine

	Preparation No.	Control	In presence of 3×10^{-4} M Nalorphine	Significance of the change
K_m (10^{-4} M)	1	6.22	18.21	
	2	8.25	19.40	
	3	4.63	42.28	
	4	3.33	27.58	
	Mean	5.61 ± 1.83	27.87 ± 9.62	$p < 0.01$
V (μmole/g liver/hr)	1	16.95	10.24	
	2	23.02	7.78	
	3	19.32	11.45	
	4	18.76	10.08	
	Mean	19.51 ± 2.20	9.89 ± 1.32	$p < 0.01$

Both K_m and V, determined in the presence of 3×10^{-4} M nalorphine, were significantly different from the constants determined in the absence of the inhibitor.

(2) The Inhibition of T2636 N-Demethylation

The data for the inhibition of T2636 metabolism by nalorphine are shown in Table II.

TABLE II

The Inhibition of T2636 N-Demethylation by Nalorphine

	Preparation Number	Control	In Presence of 3×10^{-4} M Nalorphine	Significance of the change
K_m (10^{-4} M)	1	2.66	3.87	0.05>p>0.02
	2	2.89	3.60	
	3	1.72	2.38	
	4	1.83	4.23	
	Mean	2.28 ± 0.51	3.52 ± 0.69	
V (μmole/g liver/hr)	1	5.88	3.71	0.6>p>0.5
	2	4.63	3.88	
	3	5.10	4.73	
	4	4.28	4.24	
	Mean	4.97 ± 0.58	4.64 ± 0.68	

V in the presence of nalorphine was lower than in the control although the significance of the difference was doubtful (0.6>p>0.5) as determined by the "Student" T test. The difference was shown to be significant by the method of Wilkinson (14) in which the four pairs of values of V and their standard errors were computed and the corresponding values tested for the significance of the difference. The relevant "t" values of these four significance tests were combined to support the conclusion that nalorphine significantly ($p<0.05$) depresses V. K_m determined in the presence of nalorphine was significantly greater than the control value.

(3) The N-Dealkylation of Nalorphine

The rates of N-dealkylation of morphine and nalorphine at a substrate concentration of 5×10^{-4} M were determined for each of the microsomal preparations after storing overnight at -40^{0}C. The morphine was N-demethylated at 10.12 ± 1.52 μmole/g liver/hour and nalorphine was

N-dealkylated at 18.75 ± 3.48 μmole/g liver/hour. These results suggested that the inhibitor concentration might decrease at almost twice the rate of the substrate during the incubation therefore, the extent of the inhibition of the demethylation of morphine (0.5 mM) produced by 0.1mM nalorphine was determined after various periods of incubation. The results are illustrated in Figure 1, where it is seen that the percentage inhibition decreases as the incubation proceeds.

DISCUSSION

If an inhibitor combines with the active centre of an enzyme, it must compete with the substrate for that site. This is competitive inhibition and the Michaelis Constant (K_m) is increased whilst the maximum velocity of the reaction (V) is unaltered. If, on the other hand, the inhibitor combines with the enzyme at a site removed from the active centre, there is no competition with the substrate. This is non-competitive inhibition, K_m is unchanged and V is decreased. However, K_m and V may both change and the inhibition is then neither wholly competitive nor wholly non-competitive.

Analysis of the present data for the demethylation of morphine (Table 1) shows that, in the presence of nalorphine, K_m is increased and V decreased and thus the inhibition is neither entirely competitive nor non-competitive.

Only two studies of the inhibitory action of nalorphine on morphine N-demethylation have been published previously. The first was by Axelrod and Cochin (6). Their data, in the form of the Lineweaver and Burk equation, are shown in Figure 2A, together with data from one of the experiments reported in this paper for comparison (Figure 2B). Only the part of the graph in full lines was published. Axelrod and Cochin concluded that nalorphine was a non-competitive inhibitor although they suggested that the reaction might be "an example of a slow pseudo-irreversible inhibition mimicking non-competitive inhibition but occurring at the same site".

However, if the curves are extrapolated backwards (broken lines in Figure 2A) they do not intersect on the $^1/S$ axis and the K_m of the reaction was changed in the presence of the inhibitor. The data of Axelrod and Cochin are, in fact,

consistent with those reported in the present paper, namely that the inhibitory action of nalorphine is not wholly non-competitive.

The second study was by Elison, Elliott, Look and Rapoport (7). They used the method of Hunter and Downs (15) to determine the nature of the inhibition. In this method the ratio of inhibited to uninhibited reaction velocities is inserted into the Michaelis-Menten relationships to give the following equations:

$$\left[\frac{Vi}{V-Vi}\right] = Ki + \frac{Ki}{K_m} \cdot S$$

for competitive inhibition and

$$\left[\frac{Vi}{V-Vi}\right] = Ki$$

for non-competitive inhibition, where V is the velocity of the reaction in the absence of inhibitor, Vi the velocity in the presence of inhibitor, I the initial concentration of the inhibitor, S the initial concentration of the substrate, Ki the inhibitor constant and K_m the Michaelis Constant. Thus the plot of $\left[I. \frac{Vi}{V-Vi}\right]$ against S has zero slope in the non-competitive case and a slope of $\frac{Ki}{K_m}$ in the competitive. The data of Elison et al are shown in Figure 3A and the data from the present experiment in Figure 3B. There is no doubt that Elison's data indicates non-competitive inhibition whereas the data in Figure 3B shows that the inhibition depends in some way upon the substrate concentration.

Using deuteromorphine ($M\text{-}NCD_3$) Elison et al found further evidence that the inhibition of morphine demethylation by nalorphine was not competitive. The N-demethylation of deuteromorphine, which they showed to have a higher K_m than morphine, was inhibited less strongly by nalorphine than that of morphine. This result is not consistent with competitive inhibition since, if this were the case, the N-demethylation of deuteromorphine would have been inhibited more strongly because it has a lower affinity for the enzyme than morphine. In as much as one experiment differed significantly from the mean inhibited values quoted (by more than four standard deviations) it is difficult to assess the importance of these observations.

The values of K_m and V for the microsomal N-demethylation of morphine, obtained in the three laboratories, are summarised in Table III.

TABLE III

THE VALUES OF K_m and V, OBTAINED IN DIFFERENT LABORATORIES, FOR THE N-DEMETHYLATION OF MORPHINE

Laboratory	K_m (10^{-4} M)	V (μmole/g liver/hour)
Leadbeater and Davies	5.61 ± 1.83**	19.51 ± 2.20 /
Elison et al.*	3.95 ± 1.44**	23.55 ± 11.75 /
Axelrod and Cochin (obtained from Figure 1A)	3.92	3.13

* Elison et al. quote V = 0.589 μmole HCHO/15 min. It is not clear whether this refers to the whole incubation mixture (10 ml) or the volume of mixture (2.0 ml) used for the actual assay. However, in Figure I of their paper they show that, with an initial concentration of 5×10^{-4} M morphine, 1.05 μmole HCHO were produced per 500 mg liver in 15 minutes. Therefore, it has been assumed that their V data refers to HCHO formed per 2.0 ml assay since this makes their V consistent with the data of their Figure 1.

** These values are not significantly different ($0.2 > p > 0.1$).

/ The values are not significantly different ($0.7 > p > 0.6$).

It may be seen that the values of K_m agree satisfactorily and hence the differences in the experimental technique employed did not modify the enzyme. However, the value of V obtained by Axelrod and Cochin was about one sixth the values reported by the other two groups of workers. It is possible that this difference is due to the different strains of rats which have been employed (Axelrod and Cochin used black NIH animals, Elison et al. Long-Evans rats and in these experiments albino rats of the Porton strain, which is derived from the Wistar strain, were used) since Adler, Elliott and George (16) have reported differences in the metabolism of morphine by different strains of rats.

In view of the apparently inexplicable discrepancy between the data presented here and by Axelrod and Cochin and those of Elison et al. the question arose as to whether the Michaelis-Menton theory (in the form of the Lineweaver-Burk and Hunter-Downs equations) could be applied to the data. These equations require that the inhibitor shall be unchanged during the reaction (17). In the enzyme preparations used, nalorphine was, in fact, metabolised at 18.75 μmole/g liver/hour, or 1.85 times faster than the substrate morphine, confirming the observation of Axelrod and Cochin (6) who reported a relative rate of 2.3 for the dealkylation of the two compounds. If this is so the extent of the inhibition should decrease during the incubation as the ratio of inhibitor to substrate decreases. This does, in fact occur, as may be seen from Figure 1 and the simple Michaelis-Menton equations are not applicable to this enzyme system.

The 10,000 x g supernatant of a liver homogenate, which was used as the enzyme system in the work discussed in this paper, contains the microsomal and soluble fractions of the liver cell and is probably contaminated with sub-mitochondrial particles and mitochondrial enzymes. In order to confirm that the data reported applies to the microsomal enzymes and is not modified by the presence of other cellular components, the experiments were repeated with microsomes which were isolated and washed twice in buffer by successive centrifuging at 100,000 x g for 60 minutes. In these experiments NADP and the soluble fraction, which contains enzymes and substrates capable of reducing it, were replaced by 5×10^{-4} M $NADPH_2$. The results obtained were similar to those reported above except that V was reduced, this was probably due to inactivation of the enzyme during the isolation process. Thus, the kinetic data reported with the crude 10,000 x g supernatant applies to the purified microsomal fraction.

To determine the inhibitory action of nalorphine unequivocally the enzyme systems, present in the microsomal fraction of the liver cell, which are involved in the N-dealkylation of morphine and nalorphine should be separated. Until these definitive experiments have been performed the hypotheses of Beckett, Casy and Harper (2) and of Axelrod (3) may neither be supported nor challenged on the basis of the inhibitory action of nalorphine on the liver microsomal enzyme system which N-demethylates morphine <u>in vitro</u>.

In the presence of nalorphine the K_m and V of the N-demethylation of T2636 were both changed significantly (Table II). Thus the inhibition was apparently neither competitive nor non-competitive, i.e. the data for the inhibitory action of nalorphine on T2636 metabolism is similar to that on morphine metabolism. This conclusion supports the thesis (9) that morphine and T2636 produce their biochemical effects by similar mechanisms.

ACKNOWLEDGMENT

We thank Mr. S. Peto, Head of the Statistical Section of The Microbiological Research Establishment, Porton Down, for valuable discussions on the statistical evaluation of the data.

Miss F.A. Randall gave skilled technical assistance in this work and Mr. D. Robinson performed the study with the washed microsomal system.

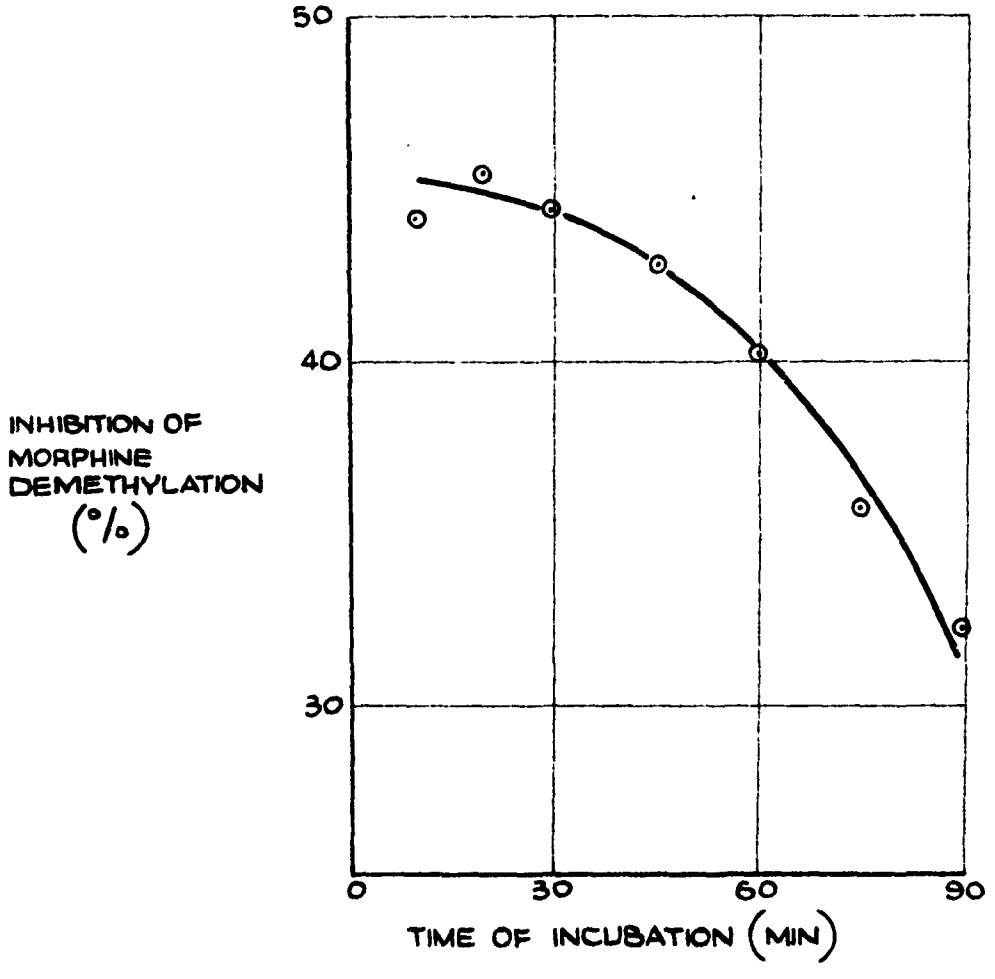
(Sgd.) D.R. Davies,
Head, Biochemistry Section.

(Sgd.) W.S.S. Ladell,
Assistant Director (Medical).

LL/DRD/JMO

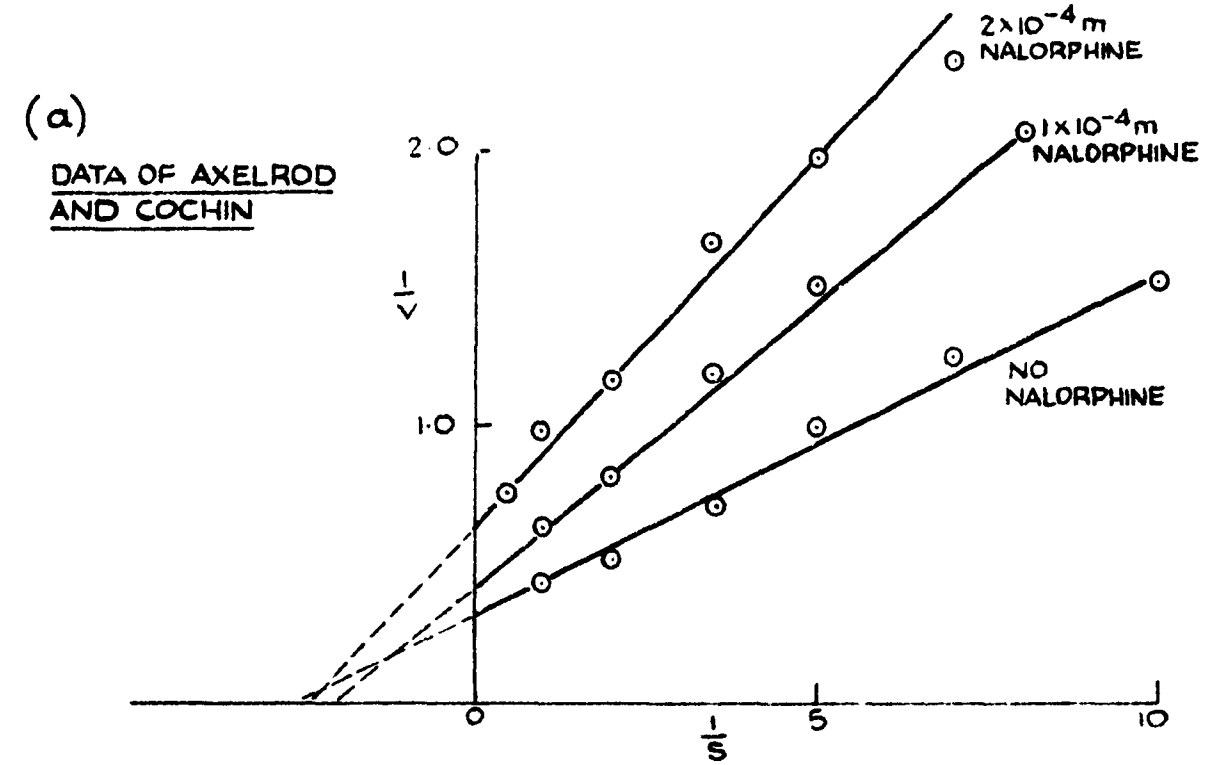
BIBLIOGRAPHY

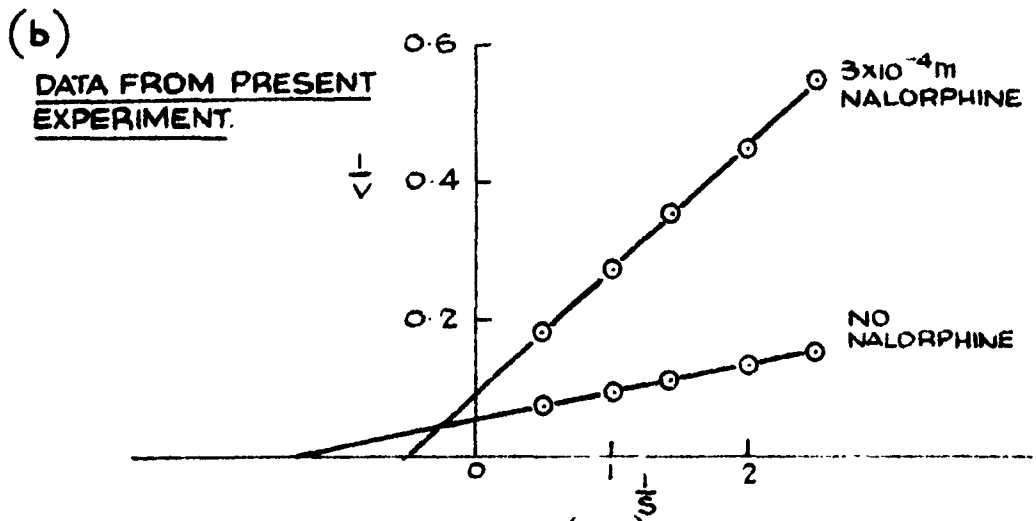
1. Unna, K. (1943). J. Pharmacol. Exp. Ther. 79, 27.
2. Beckett, A.H., Casy, A.F. and Harper, N.J. (1956). J. Pharm. Pharmacol. 8, 874.
3. Axelrod, J. (1956). Science 124, 263.
4. Cochin, J. and Axelrod, J. (1959). J. Pharmacol. Exp. Ther. 125, 105.
5. Way, E.L. and Adler, T.K. (1960). Pharmacol. Rev. 12, 383.
6. Axelrod, J. and Cochin J. (1957). J. Pharmacol. Exp. Ther. 121, 107.
7. Elison, C., Elliott, H.W., Look, M. and Rapoport, H. (1963). J. Med. Chem. 6, 237.
8. Hollyhock, W.M., Callaway, S. and Ladell, W.S.S. (1963). P.T.P.835.
9. Leadbeater, L. (1964). P.T.P.881.
10. Axelrod, J. (1956). J. Pharmacol. Exp. Ther. 117, 322.
11. Umbreit, G.R. (1961). Anal. Chem. 33, 1572.
12. Lineweaver, H. and Burk, D. (1934). J. Amer. Chem. Soc. 56, 568.
13. Fisher, R.A. (1925). "Statistical Methods for Research Workers", Oliver and Boyd, London.
14. Wilkinson, G.N. (1961). Biochem. J. 80, 324.
15. Hunter, A. and Downs, C.E. (1945). J. Biol. Chem. 157, 427.
16. Adler, T.K., Elliott, H.W. and George, R. (1957). J. Pharmacol. Exp. Ther. 120, 475.
17. Dixon, M. and Webb, E.C. (1958). "The Enzymes", Chapt. IV. Longmans, Green and Co. Ltd., London.



THE CHANGE IN THE EXTENT OF THE INHIBITION OF MORPHINE DEMETHYLATION BY NALORPHINE DURING THE INCUBATION PERIOD.

FIG.I.

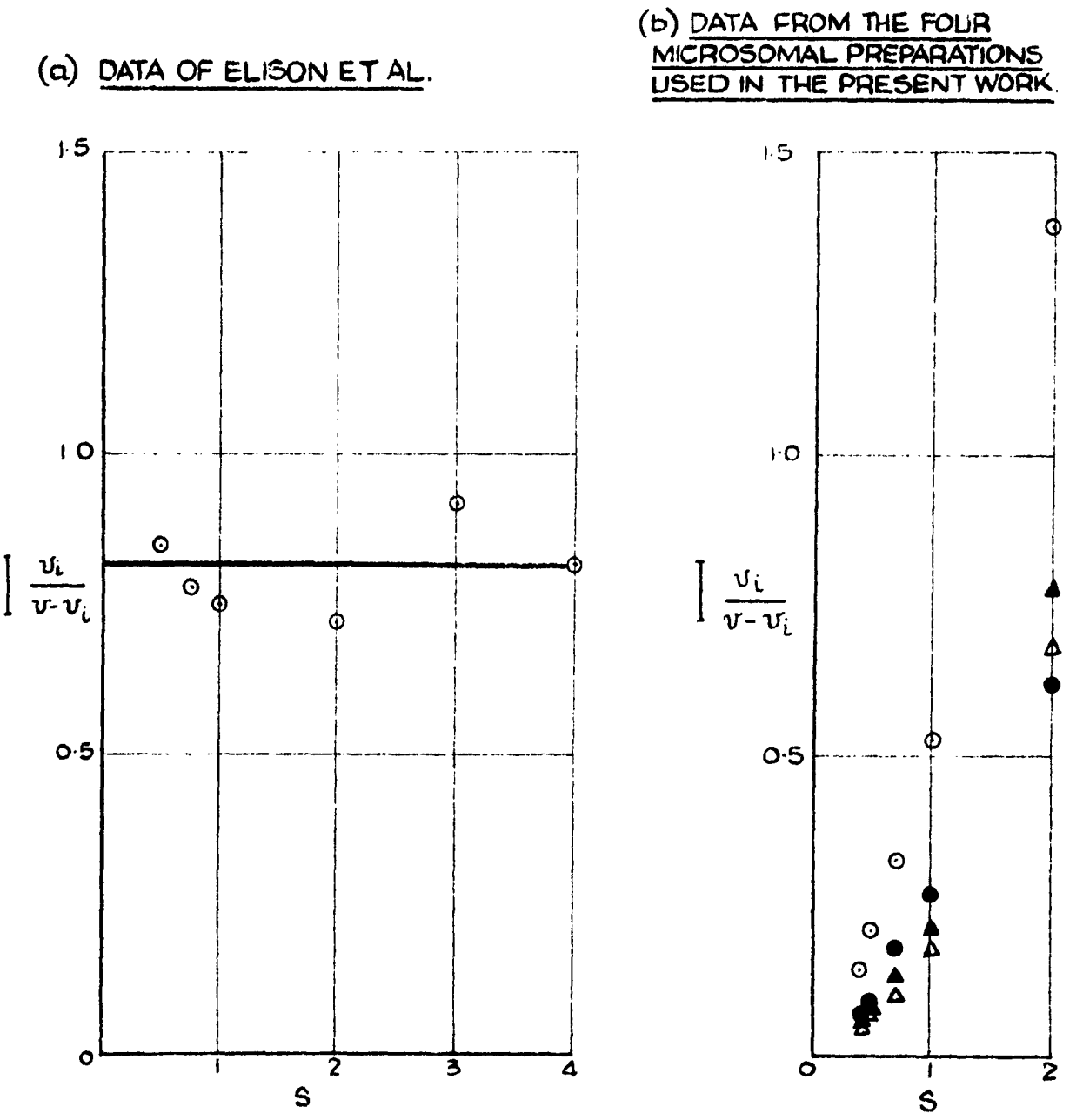




S = MORPHINE CONCENTRATION (mM)
V = μ MOLES HCHO PRODUCED PER gm LIVER PER HOUR.

THE LINEWEAVER AND BURKE PLOT.

FIG. 2.



THE HUNTER AND DOWNS PLOT.

FIG. 3.

SECRET

DISTRIBUTION TO P.T.P. 900

ARMY DEPARTMENT

1 Chief Scientist (Army)
2 DCA(A)
3-6 DBCD (4 copies)
7 AEP 4
8 DA Path
9 D/AORE
10 R Army Military College (Col. M.W. Whitcher)

R. & D. ESTABLISHMENTS

87-116 CDEE
117 MRE

ADVISORY BODIES

Chemical Defence Advisory Board

11 Prof S. Tolansky
12 Prof R.B. Fisher
13 Dr J.M. Barnes
14 Dr O.G. Edholm
15 Prof D.H. Hey
16 Prof B.J. Mason
17 Prof H.N. Rydon
18 Mr A.L. Stuchbery
19 Prof R.H.S. Thompson
20 Prof D.D. Woods
21 Prof A. Wilson

Biology Committee

22 Prof M. Weatherall
23 Prof E. Boyland
24 Prof K.W. Donald
25 Sir Charles Lovatt Evans
26 Dr J.D.P. Graham
27 Prof W.T. Irvine
28 Dr R.D. Keynes
29 Prof Sir Aubrey Lewis
30 Sir Rudolph Peters
31 Dr J.R. Squire
32-37 Secretariat SAC (6 copies)

NAVY DEPARTMENT

38 MDG (N)

AIR FORCE DEPARTMENT

39 DDOR (RAF)
40 D. Hygiene & Research

MINISTRY OF AVIATION

41-42 TIL (2 copies)

MINISTRY OF HEALTH

43 Dr. D. Thomson

HOME OFFICE

44 CSA's Dept (Attn Dr J. McAulay)

UK HIGH COMMISSION, OTTAWA

45 Senior Army Liaison Officer

BRITISH DEFENCE STAFFS, WASHINGTON

46-52 Dr D. Downing, DR Staff (7 copies)

OVERSEAS (Through TIL)

Australia

53-55 Defence Standard Laboratories (3 copies)
56 Senior Representative, Dept of Supply
57 Army Staff (UK)
58 RAAF (Tech Section)

Canada

59-60 Chairman, Defence Research Board (2 copies)
61-62 Defence Research Chemical Laboratories, Ottawa (2 copies)
63 Suffield Experimental Station

U.S.A.

64-79 Reading Panel (16 copies)
80-86 US Liaison Officer, Porton (7 copies)

SECRET

Information Centre
Knowledge Services
[dstl] Porton Down,
Salisbury
Wilts
SP4 0JQ
22060-6218
Tel: 01980-613933
Fax 01980-613970

Defense Technical Information Center (DTIC)
8725 John J. Kingman Road, Suit 0944
Fort Belvoir, VA 22060-6218
U.S.A.

AD#: AD355396

Date of Search: 30 May 2008

Record Summary: WO 189/415
Title: Inhibition of Liver Microsomal N-Demethylation of Morphine and T.2636 by N-Ally Normorphine and its Pharmacological Implications
Availability Open Document, Open Description, Normal Closure before FOI Act: 30 years
Former reference (Department) PTP 900
Held by The National Archives, Kew

This document is now available at the National Archives, Kew, Surrey, United Kingdom.

DTIC has checked the National Archives Catalogue website (http://www.nationalarchives.gov.uk) and found the document is available and releasable to the public.

Access to UK public records is governed by statute, namely the Public Records Act, 1958, and the Public Records Act, 1967.
The document has been released under the 30 year rule.
(The vast majority of records selected for permanent preservation are made available to the public when they are 30 years old. This is commonly referred to as the 30 year rule and was established by the Public Records Act of 1967).

This document may be treated as UNLIMITED.